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Protein kinase C beta (PKC beta): normal functions and diseases.
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Activation Mechanisms of Protein Kinase C: Maturation, Catalytic Activation, and Targeting

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The biological function of protein kinase C (PKC) depends on its catalytic activity and spatial localization. Its catalytic competence and localization in the resting state are regulated by serine/threonine phosphorylations, *i.e.*, "maturation." Upon stimulation of various receptors, PKC is catalytically activated by several activators including diacylglycerol. In addition, PKC often translocates to particular subcellular compartments including the plasma membrane and Golgi complex, and such translation is here referred to as "targeting." In short, the physiological function of PKC is controlled by the three events: maturation, catalytic activation, and targeting. Catalytic activation and targeting contribute to temporal, spatial, and isotype-specific regulation of PKC. This review summarizes the evidence for the role of these three events in the isotype-specific activation of PKC, with particular emphasis on catalytic activation and targeting by lipid mediators.

Key words: catalytic activation, lipid mediator, maturation, targeting.

1. Overview of activation mechanism

In addition to the PKC-related kinases (PKD and PKN), at least 10 isotypes of mammalian PKCs have been identified and divided into three groups: conventional PKC (cPKC: α, βI, βII, and δ-isotypes), novel PKC (nPKC: δ, ε, η, θ-isotypes) and atypical PKC (aPKC: ζ and ι/λ-isotypes) (*1*, *2*, also see the prologue in this minireview series). The regulatory domain of cPKCs contains two conserved modules, C1 and C2 domains. Unlike cPKCs, the C2 domain is missing in nPKCs, and aPKCs lack the entire C2 domain and one cysteine-rich loop in the C1 domain.

In spite of the large number of isotypes, their low substrate-specificity and the expression of multiple isotypes in the same cell, each PKC seems to have its own function. How is this isotype-specificity controlled? The original activators of PKC were phosphatidylserine, calcium ion and diacylglycerol (*3*). Additional lipid mediators, including fatty acids and lysophospholipids, have recently been shown to enhance the catalytic activity of PKC (reviewed in Ref. *4*). Since these mediators differentially affect the catalytic activity of each PKC, they represent one mechanism for isotype-specific regulation. The ability of different physiological stimuli to selectively translocate isotypes to distinct subcellular compartments (reviewed in Ref. *5*) is an additional mechanism by which cells can regulate where, when, and which PKC acts. This stimulus-dependent targeting of PKC has been visualized by live imaging using green fluorescent protein (GFP)–conjugated PKCs, and the different targeting leads to distinct cellular responses. In addition, Newton and coworkers showed that serine/threonine phosphorylations, which they define as "maturation," are necessary for PKC's catalytic competence and correct subcellular localization (reviewed in Ref. *2*). Together, these studies demonstrate that the isotype-specific physiological function of PKC is regulated by the three events: maturation, catalytic activation, and targeting (Fig. 1).

In the following sections, we review the literature supporting a role for maturation, catalytic activation and targeting in the selective activation of the mammalian PKCs.

2. Maturation

Using PKCβII as a model, Newton *et al.* have proposed the following pathway for maturation (*2*). The earliest translation products are unphosphorylated, cytoskeletally-associated proteins. The initial step in maturation is phosphorylation of Thr500 in the activation loop by phosphoinositide-dependent kinase 1 (*6*). This aligns the active site, which permits subsequent auto-phosphorylation on Thr641 in the turn motif and Ser660 in the hydrophobic motif (*7*). The phosphorylation of Thr in the activation loop is essential for maturation, because the T500A mutation of PKCβII does not become phosphorylated and accumulates in the detergent-insoluble fraction (*8*). However, once the C-terminal serine and threonine are phosphorylated, the enzyme assumes its mature conformation, and subsequent dephosphorylation of Thr500 does not alter its ability to be activated and/or to translocate. In contrast, dephosphorylation of the Ser/Thr in the turn motif abolishes kinase activity, suggesting that these residues are critical for activation (*7*).

These three sites are conserved among the PKC isotypes (Fig. 2), suggesting that all PKCs mature *via* a similar pathway. The exceptions are aPKCs and PKCδ. The aPKCs have a glutamic acid instead of serine or threonine in the hydrophobic motif. In PKCδ, Thr505 in the activation loop is not necessary for its catalytic activity, because the negative charge in the activation loop is compensated by Glu500 (*9*). Although a different amino acid, the acidic nature of the substitution is consistent with Newton's model of maturation (*2*). This series of phosphorylations is required for

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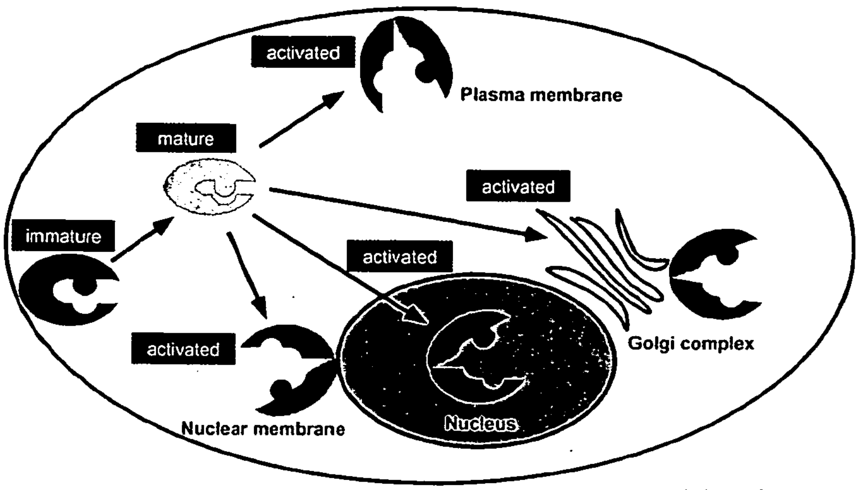
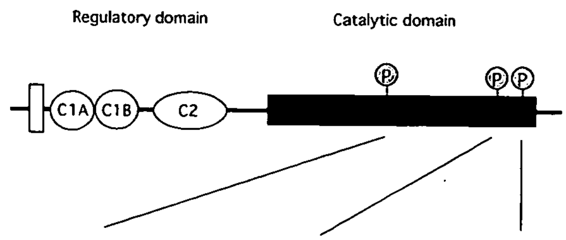


Fig. 1. **Overview of PKC activation.** The physiological function of PKC is controlled by three events: maturation, catalytic activation and targeting. Newly translated "immature" PKC (shown in navy) cannot be catalytically activated. It is serine/threonine-phosphorylated at three distinct sites, thus maturing into a form that is localized in the cytosol (light blue) and is sensitive to physiological stimuli. Upon stimulation of various receptors, this "mature" PKC can be catalytically activated by several activators and targeted to specific subcellular compartments including plasma membrane, Golgi complex, nuclear membrane and nucleus (shown in red).



	Activation loop	Turn motif	Hydrophobic motif
α	497T	638T	657S
βI	500T	642T	661S
βII	500T	641T	660S
γ	514T	655T	674T
δ	505T	643S	662S
ε	566T	710T	729S
η	512T	655T	674S
θ	538T	676S	695S
ζ	410T	560T	(579E)
ι/λ	403T	555T	(574E)

Number shows position of serine / threonine.

Fig. 2. **Conservative serine/threonine residues in the activation loop, turn motif, and hydrophobic motif of 10 mammalian PKCs.**

the catalytic competence and correct intracellular localization of PKCs in the resting state. Therefore, it represents one of the rate-limiting steps for PKC activation.

3. Catalytic activation

3-1. Phosphatidylserine. Phosphatidylserine (PS) is necessary for catalytic activity of all PKCs. It is thought to bind to either the C1 or C2 domains but the specific binding site has not been identified. The crystal structure of the C2 domain of PKCα in the presence of Ca^{2+} and PS reveals that a short chain of PS is coordinated to the C2 domain (*10*). In contrast, the binding of PS to the C1B domain of PKCβII has also been reported (*11*). Additional studies are necessary to determine how PS interacts with each isotype.

3-2. Calcium ions. Calcium ions (Ca^{2+}) regulate the activity of the cPKCs via the C2 domain. The Ca^{2+}-binding, C2 domain of the cPKCs is homologous to that of synaptotagmin. Based on crystallographic analysis of the synaptotagmin C2 domain in the presence and absence of Ca^{2+} (*12*), it appears that Ca^{2+}-binding changes the conformation of the C2 domain. Although this has not yet been demonstrated for PKC, by analogy one can propose that Ca^{2+}-binding to the cPKC-C2 domain also induces a conformational change (*13, 14*). As the C2 domains of PKCα and β have been crystallized (*15, 16*), it is likely that these studies are under way.

In addition to its putative role in modulating the conformation of PKC, Ca^{2+} also increases the affinity of the enzyme for PS. The Ca^{2+}-induced increase in the affinity of PKC-C2 domain for PS was suggested by initial studies using lyposomes (*17*), and directly demonstrated by Newton and coworkers (*18, 19*). Interestingly, they also suggested that each isotype of cPKCs is differentially regulated by calcium (*19*).

3-3. Lipid mediators. *3-3a. Diacylglycerol (DAG) and phorbol ester.* DAG and phorbol ester activate the cPKC and nPKCs by binding to the C1 domain (*1, 20*). The C1 domain of the cPKC and nPKCs has two cysteine-rich domains (C1A and C1B), each containing ~50 amino acids, including six cysteine and two histidine residues arranged in a zinc finger motif. The aPKCs lack one of the cysteine-rich domains and thus are insensitive to activation by these compounds. The C1B domain of PKCδ has been crystallized and shown to contain a phorbol ester–binding pocket (*21*).

Two types of DAG appear to be important for the physiological activation of PKC. One is rapidly produced from phosphatidylinositol 4,5-bisphosphate (PIP_2) by phospholipase C upon stimulation of G protein-coupled receptors. The other is thought to result from hydrolysis of phosphatidylcholine (PC). This latter DAG production occurs slowly and is more sustained. In addition to the temporal difference, the fatty acid composition of the DAG derived from PC differs from that released from PIP_2. Interestingly, PKC isotypes are differentially sensitive to the fatty acid composition of DAG (*22*). 1-Steroyl-2-arachidonoyl-*sn*-glycerol (SAG) stimulates PKCα and δ more effectively than do 1-steroyl-2-docosahexaenoyl-*sn*-glycerol (SDG) and 1-steroyl-2-eicosapentaenoyl-glycerol (SEG). In contrast, activation of PKCβI by SDG and SEG is higher than that by SAG. Thus, the composition of the DAG and its temporal release may regulate isotype-selective activation.

3-3b. Fatty acid. Fatty acids are known to activate PKC in an isotype-specific manner (*4*). For example, saturated

fatty acids having a carbon chain length of between C13 and C18 activate PKCα, β, γ, and ε *in vitro* (*23*); and this effect is enhanced by the co-presence of DAG (*23, 24*). Similarly, unsaturated fatty acids such as arachidonic and oleic acid stimulate PKCγ and ε (*23, 24*). In contrast, δPKC is not activated by saturated fatty acids and is inhibited by arachidonic acid (*23*).

3-3c. Ceramide. Ceramide, which is produced from sphingomyelin by sphingomyelinase, is also a modulator of PKC activity, although its effects are controversial. Bourbon et al. reported that ceramide directly activates PKCζ (*25*), while Huwiler *et al.* failed to show direct binding of radiolabeled ceramide to PKCζ (*26*). Additionally, ceramide at low concentrations activates PKCα (*26*), but at higher concentration it inhibits or has no effect on its kinase activity (*26, 27*).

3-3d. Other lipids. Cholesterol sulfate (CS) is a unique lipid in epidermal tissue and a metabolite of cholesterol formed during differentiation of squamous epithelium. It has been shown to selectively activate PKCη (*28*). Phosphatidic acid and lysophospholipids such as lysophosphatidylcholine (LPC) also increase PKC activity (*4*). LPC enhances the activity of cPKCs but not nPKCs (*29*). Other lipids which modify PKC activity include phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 3,4,5-triphosphate. These compounds activate PKCε, η, and ζ, but not PKCα, β, βII, and γ (*30, 31*).

Taken together, these data demonstrate that DAG is not the only lipid with PKC-activating properties. Other lipids exhibit selective effects on different PKC isotypes, thus providing an intriguing mechanism for isotype selective activation.

3-4. Tyrosine phosphorylation. In addition to activation by lipid mediators, the catalytic activity of PKC can be modulated by tyrosine phosphorylation; PS is not required for this activation. (The details of this activation will be described in Kikkawa's review in this series.) Briefly, Konishi *et al.* reported that hydrogen peroxide induces tyrosine phosphorylation of PKCα, βI, δ, γ, and ζ, resulting in enhanced enzyme activity (*32*). In contrast, Denning *et al.* demonstrated that the enzymatic activity of PKCδ is inhibited by tyrosine phosphorylation (*33*). The explanation for this apparent paradox is not clear but may be due to differences in cell type or mode of activation.

4. Targeting

Kraft *et al.* (*34*) reported for the first time that PKC translocates from the soluble fraction to the particulate fraction in response to phorbol 12-myristate 13-acetate (PMA). Subsequent biochemical and immunochemical approaches using isotype-specific antibodies have revealed that physiological stimulation, including that by thyrotropin-releasing hormone, also induces translocation of PKC (*35*, reviewed in Refs. *5* and *36*) and some responses are isotype-specific: α-thrombin and platelet-derived growth factor induce translocation of PKCα, but not PKCε and ζ, to the nucleus in IIC9 and Swiss 3T3 cells (*37, 38*). More recently, GFP technology has allowed the dynamic movement of PKC to be visualized in living cells and has confirmed a remarkable diversity in PKC targeting as described in this section. These studies indicate the importance of targeting in regulating the physiological and isotype-specific function of PKC.

At least two domains, C1 and C2, are thought to be involved in targeting (*1, 2, 14*). It is hypothesized that Ca^{2+}-binding to the C2 domain changes its conformation and increases its affinity for PS, resulting in the membrane targeting. In fact, Ca^{2+} ionophores induce translocation to the plasma membrane of GFP fusion proteins containing the PKCγ-C2 domain or full-length PKCγ (*13, 39*), or PKCα (40). In these translocations, Ca^{2+} mobilization is synchronized with the membrane targeting of the PKCs (*13, 40*).

The C1 domain contains phorbol ester–binding sites (*20, 21, 47*). This is evident from studies demonstrating that PMA induces irreversible membrane targeting of the GFP-tagged C1 domain of PKCγ (*41*) or full length of PKCα (*40*), βII (*42*), γ (*39, 41*), δ (*43, 44*), or ε (*45*). DAG, which binds to the same site on the enzyme, causes transient translocation of PKC ε to the plasma membrane (*46*). Irie *et al.* reported that the C1B domains of the nPKCs have higher affinity for phorbol ester than their C1A domains (*47*). In contrast, the C1A and C1B domains of PKCγ have equivalent affinity (*47*). Mutation of the C1B domain of PKCδ, but not C1A, impairs PMA-induced translocation (*48*), while the C1A and C1B domains of PKCγ are equivalent in their ability to translocate to the plasma membrane in response to PMA (*41*).

Blumberg and coworkers reported that phorbol ester and related ligands induce targeting of PKCδ to different cellular membranes (*49*). Like PMA, phorbol 12,13-dioctanate and phorbol 12,13-nonanoate cause irreversible translocation to the plasma membrane. In contrast, two highly lipophilic derivatives, phorbol 12,13-dibutyrate and phorbol 12,13-dihexanoate, result in localization of PKCδ to the nuclear membrane and perinuclear region. Interestingly, the former ligands have high tumor-promoting activities, while the latter compounds are less tumorogenic. For example, a strong tumor promotor, 12-deoxyphorbol 13-tetradecanoate, stimulates PKCδ concentration at the plasma membrane, while a natural inhibitor of tumor promotion, bryostatin 1, induces the nuclear membrane targeting (*44*).

Fatty acids, as well as other mediators, induce isotype-specific translocation. Saturated fatty acids induce translocation of PKCε from the cytoplasm to the plasma membrane, while arachidonic acid translocates εPKC to the Golgi complex (*46*). Unlike PKCε, PKCζ can be translocated to the nucleus by AA (our unpublished data), but PKCδ is insensitive to this fatty acid (*50*). More recently, we demonstrated that the C1B domains of PKCδ and ε are responsible for their distinct sensitivity to AA (*45*). Using δ/ε chimeras, we have shown that a chimera of PKCδ having the C1B domain of PKCε translocates to the Golgi complex in response to AA, but a chimera of PKCε expressing the C1B domain of PKCδ does not respond to AA. However, the mechanism of the fatty acid-induced targeting is still unknown and the nature of the interaction between AA and the C1 domain of PKCε remains to be elucidated.

Like AA, ceramide also induces isotype-specific targeting. A membrane permeable analogue of ceramide translocates PKCδ and ε from the cytoplasm to the Golgi complex, but PKCα and ζ are insensitive to this treatment (*45, 51*). The same analogue activates PKCδ *in vivo via* tyrosine phosphorylation, but the ceramide-induced targeting to the Golgi complex does not depend on tyrosine phosphorylation (*51*).

Binding of ligands on receptors has isotype-specific

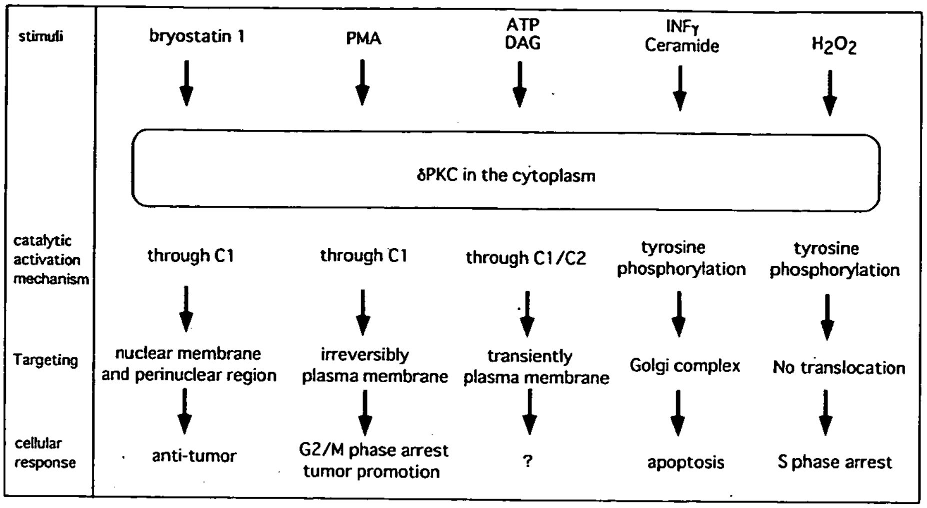


Fig. 3. **Diverse cellular response resulting from distinct targeting of δPKC.** Irreversible membrane-targeting of δPKC by PMA induces G2/M arrest or tumor promotion, while anti-tumor ligands such as bryostatin 1 translocate δPKC to the perinuclear region including nuclear membrane. Ceramide-induced targeting to the Golgi complex results in apoptosis and activation of δPKC by hydrogen peroxide treatment, which does not change the localization, increases the number of cells in S phase. ATP causes transient targeting to the plasma membrane, but the significance of this response is not known.

effects similar to those described above. Carbacol and angiotensin II induce transient translocation to the plasma membrane of α and βII PKC, respectively (*40, 42*). Similarly, PKCγ, δ, and ε are targeted to the plasma membrane in response to purinergic stimulation (*50*). We also found that INFγ stimulation, which generates ceramide, causes translocation of PKCδ to the Golgi complex, in a similar way to treatment of cells with the ceramide analogue (*51*). Additionally, PAF stimulation, which mobilizes AA in addition to DAG production and Ca^{2+} increase, localized ζPKC to the nucleus (our unpublished data).

Physiologically, targeting PKC to the plasma membrane is necessary for phosphorylation of membrane-associated substrates. This is clearly illustrated by our experiments demonstrating that PMA-induced targeting of PKCδ to the plasma membrane results in phosphorylation of MARCKS on the membrane, whereas PKCδ activation by hydrogen peroxide, which does not elicit translocation, fails to phosphorylate this substrate (*52*). Other experiments revealed that ceramide-induced targeting of PKCδ to the Golgi complex resulted in apoptosis (unpublished data), while PMA-induced activation and plasma membrane targeting of PKCδ increased the number of cells in G2/M phase (*43*). Thus, differential targeting leads to distinct cellular responses (some of which are summarized in Fig. 3).

The diversity in the number and effects of lipid mediators on PKC localization and cellular responses reflect the importance of targeting in the physiological activation and function of the different PKC isotypes. Thus, targeting plays a major role in regulating isotype-specific signal transduction.

5. Perspective and conclusion

The importance of targeting in isotype-specific activation of PKC has become apparent, but a question regarding the correlation between targeting and catalytic activation remains to be solved: does PKC translocate after it becomes catalyticaly active or is it activated after its translocation? To address this issue, PKC inhibitors and a kinase-negative mutant have been used. PMA induces translocation of PKCγ in the presence of a PKC inhibitor, staurosporine (*39*), and the kinase-dead PKC βII shows translocation, although its re-translocation is inhibited (*53*). Moreover, PKCδ can translocate to the Golgi complex without catalytic activation in response to ceramide (*51*). These results indicate that translocation of PKC is independent of its catalytic activity. However, we can not conclude that PKC becomes catalytically active after translocation, because hydrogen peroxide activates PKCδ without translocation (*51*). Development of a fluorescent substrate which will enable us to spatio-temporally visualize PKC phosphorylation would be useful to evaluate the correlation between translocation and catalytic activation.

Although the molecular mechanism of PKC targeting is not well understood, a scaffold protein such as AKAP or RACKS may determine the localization of PKC when activated (reviewed in Refs. *54* and *55*). Lipid mediators can be also candidates as targeting modulator.

In conclusion, the physiological function of PKC is regulated by maturation, catalytic activation and targeting. Catalytic activation and targeting are temporally and spatially orchestrated, contributing to isotype-specific activation of PKC under physiological conditions. In these events, lipid mediators play important roles.

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